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Ultrashort Peptides as Bifunctional Nanomaterials

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Introduction

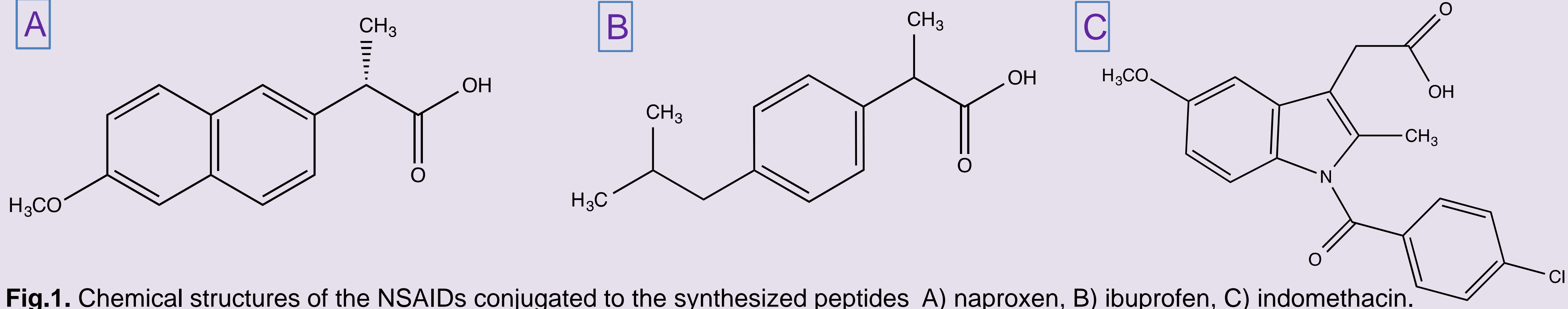
Biomaterials have contributed greatly to advances in modern medicine enhancing patients’ quality of life however they are regarded as foreign objects by the human body [1]. Their presence and sometimes trauma caused by insertion of a biomaterial triggers host inflammatory mediators. They also provide an ideal surface for bacterial attachment and biofilm formation. These can compromise biomaterial function and mechanical properties resulting in its failure or destruction of with associated negative effects on the patient and their outcomes [2].

Self-assembling ultrashort cationic peptides are an innovative form of antimicrobial hydrogels. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain and inflammation despite their systemic side effects. A combination of these may create a novel self-assembling biomaterial with dual properties- a localized antimicrobial action and ability to dampen the host immune response [3].

Gelation can be tailored to occur in response to physiological infective indicators:

- + pH
- + Enzymes
- + Temperature

Thus facilitating targeted antimicrobial and anti-inflammatory activity at the site of infection [4]. This work focuses on the development of dual acting anti-inflammatory and antimicrobial self-assembling peptides with the incorporation of clinically used NSAIDs into our previously investigated self-assembling antimicrobial peptides with -FFKK.



Results and discussion

The ability to self-assemble to form hydrogels is determined by the primary peptide structure. Naproxen and indomethacin conjugated peptides formed self-supporting hydrogels. Ibuprofen conjugates due to insufficient π - π stacking, remained as viscous solutions confirmed via viscosity measurements. Rheological analysis confirmed the hydrogels as viscoelastic with G' being one order of magnitude higher than G'' for all concentrations of peptides. FTIR further confirmed assembly with shoulders in the amide I region (1570 cm^{-1}) and characteristic peaks representative of β -sheet formation (1625 cm^{-1}) in an antiparallel arrangement (1675 cm^{-1}). Higher ordered nanofibrous assemblies were observed under cryo-SEM and TEM.

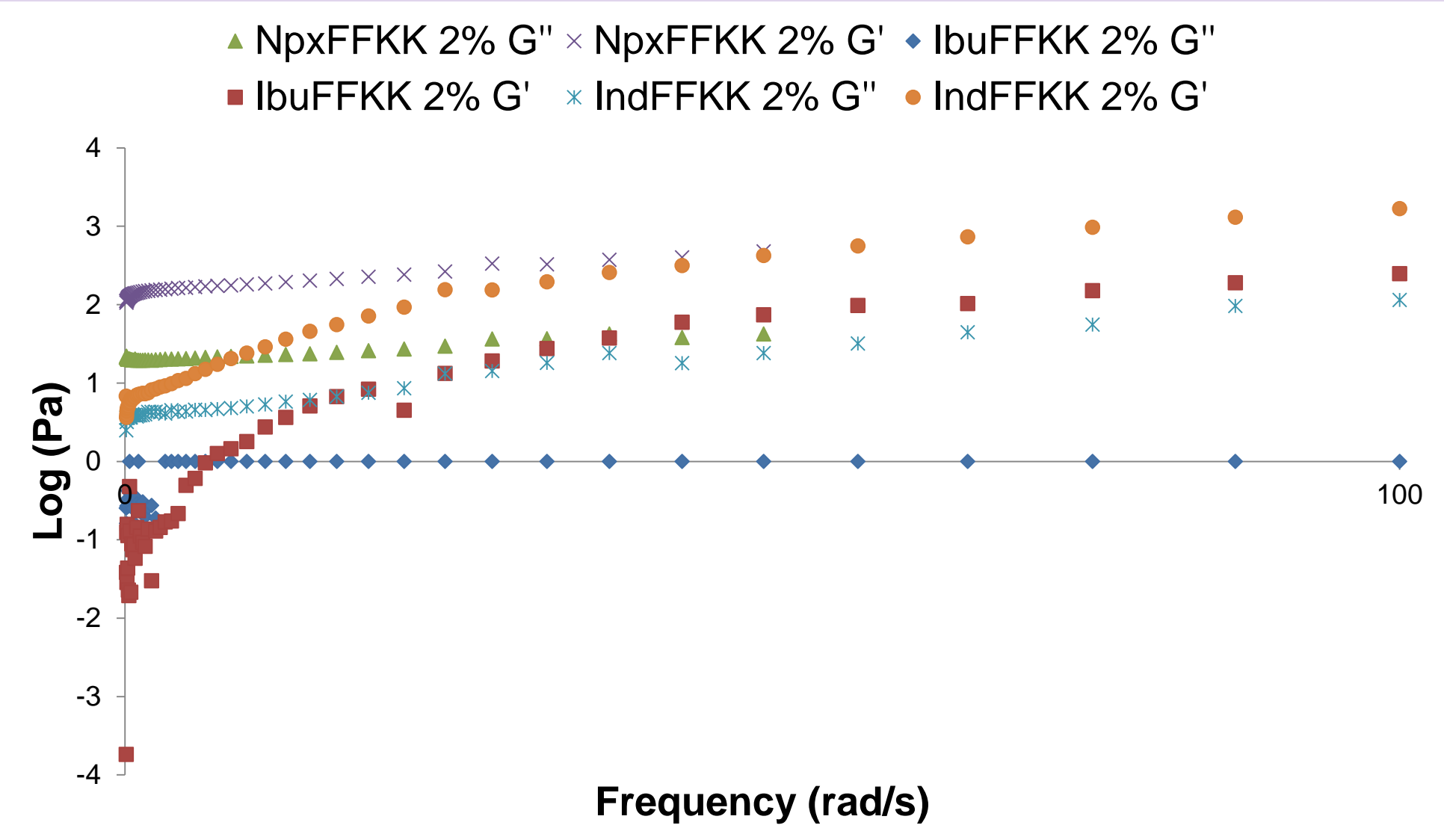


Fig.2. Frequency sweep and rheological properties for NSAID conjugates at 2% (w/v). G' = storage modulus, G'' = loss modulus

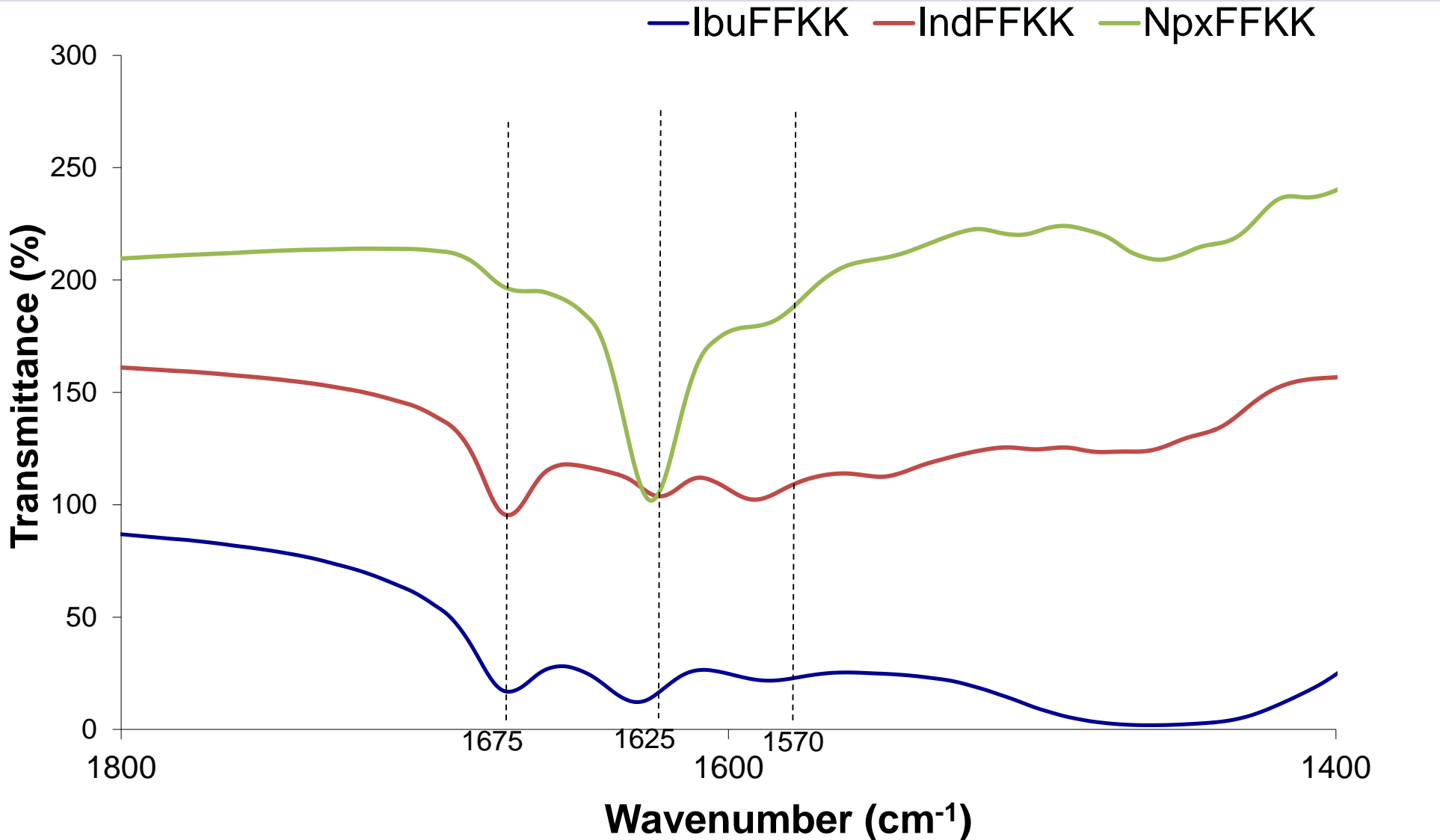


Fig.3. FTIR spectra for the NSAID conjugates at 2% (w/v).

The hydrogels proved to be relatively non-hemolytic and non-toxic *in vitro*. Strong antibiofilm activity was demonstrated by all hydrogels. The optimal reduction in percentage viable biofilm was exhibited by IndFFKK against *Staphylococcus epidermidis*. The anti-inflammatory activity of the NSAIDs was not inhibited following coupling to the peptide with hydrogels showing inhibitory activity against both COX-1 and COX-2 enzymes.

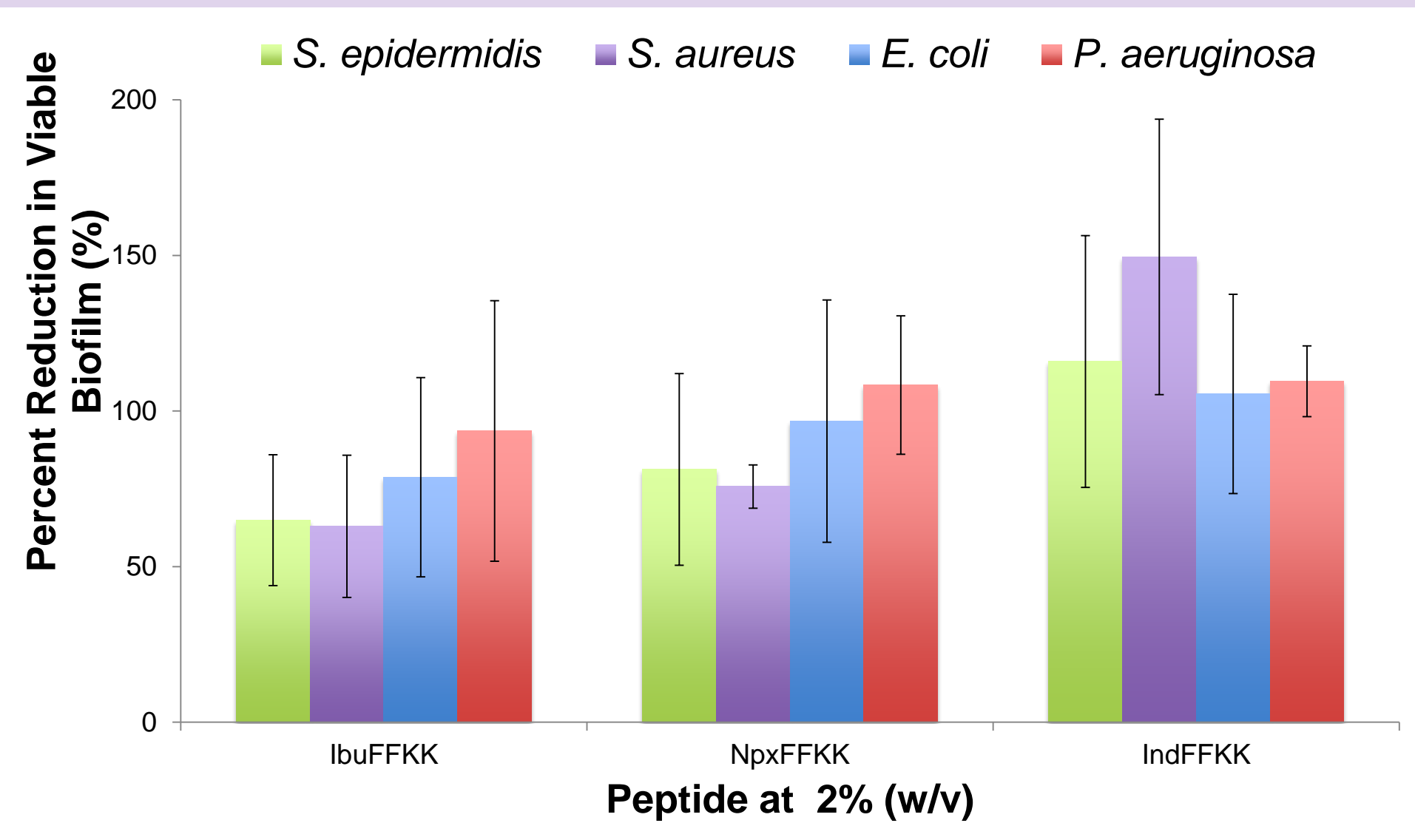


Fig.5. Percentage reduction of mature 24 h biofilms treated with 2% (w/v) NSAID conjugated hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 6 replicates.

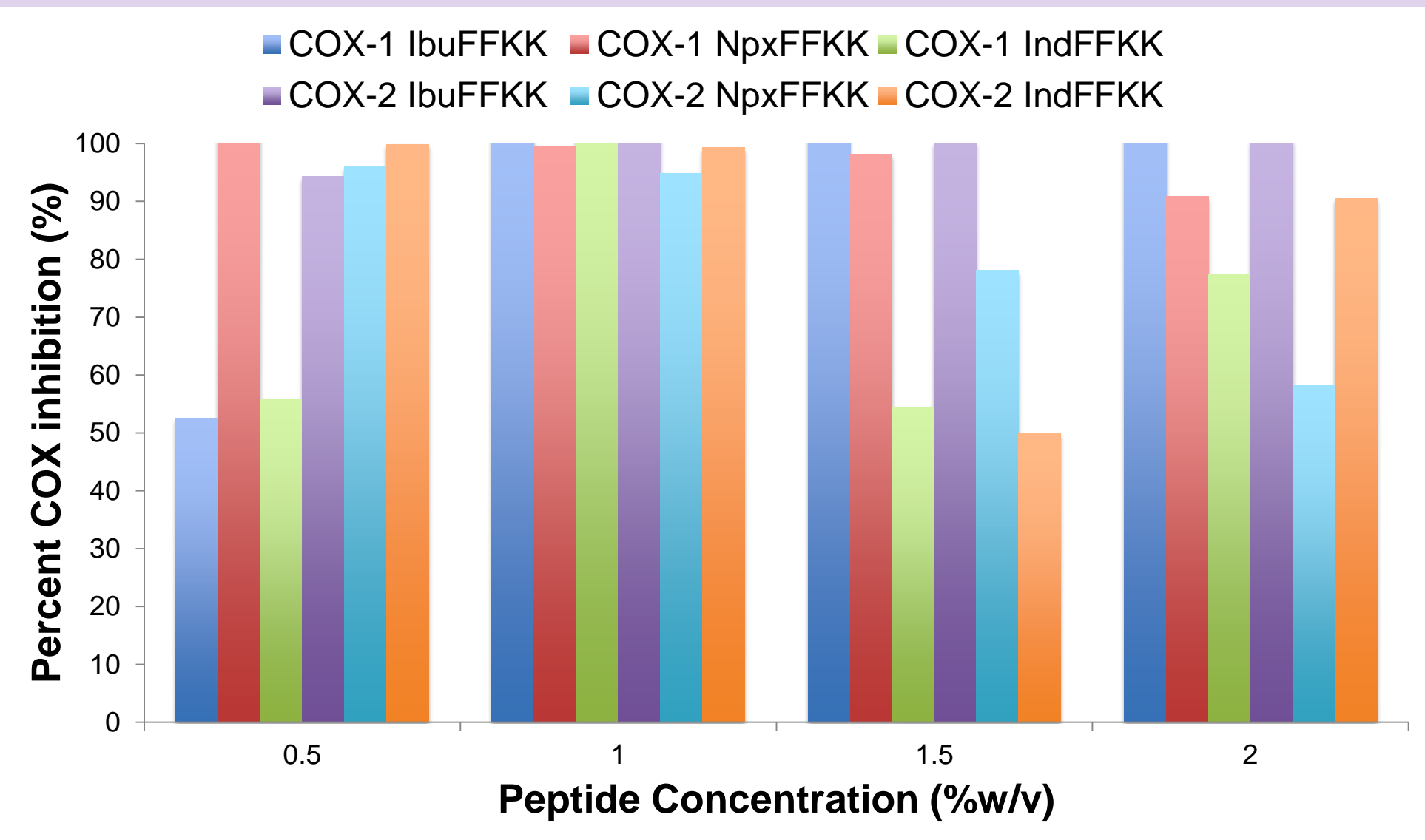


Fig.6. Percentage inhibition of COX-1 and -2 enzymes following treatment with NSAID conjugated hydrogels. Results are displayed as a mean of 3 replicates.

Methods

Diphenylalanine-dilysine peptides were synthesized on Wang resin following standard Fmoc protocols using a manual Nitrogen bubbler apparatus. The final coupling step involved addition of one of the following NSAIDs (listed below) in the same manner as to the addition of an amino acid:

- + naproxen (Npx)
- + ibuprofen (Ibu)
- + indomethacin (Ind)

Gelation was triggered by addition of sterile deionized water, raising the pH to \sim pH 9 with 1M NaOH to ensure full dissolution of the peptide. 0.5M HCl titrated the pH of the peptide solution to near physiological pH (\sim pH 7.4). Gelation was assessed after 24 hours via the inversion method.

The viscoelastic nature of the peptides was characterized by oscillatory rheology. Hydrogels with a storage modulus (G') at least one order of magnitude greater than the loss modulus (G'') were classified as being viscoelastic.

Cryo-scanning electron microscopy (cryo-SEM) and transmission electron microscopy (TEM) confirmed the presence of nanofibrous networks. This was further confirmed with evidence of beta stacking in anti-parallel arrangement determined by FTIR.

FTIR provided information regarding the secondary structure of the peptides. Hydrogels were prepared as detailed above but with deuterated solvents. Peaks were obtained from an average of 128 scans and wavelengths of $4000\text{--}400\text{ cm}^{-1}$.

Hemolytic activity was determined using equine erythrocytes and cytotoxicity against (NCTC929) murine fibroblast cells. Activity against cyclooxygenase -1 and -2 (COX-1 and -2) proved anti-inflammatory activity of the peptide hydrogels.

The antimicrobial properties of each concentration of peptide hydrogel were determined by the ability to reduce viable 24 hour biofilms of both Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 35984) and Gram-negative bacteria (*Escherichia coli* NCTC 11303 and *Pseudomonas aeruginosa* PAO1).

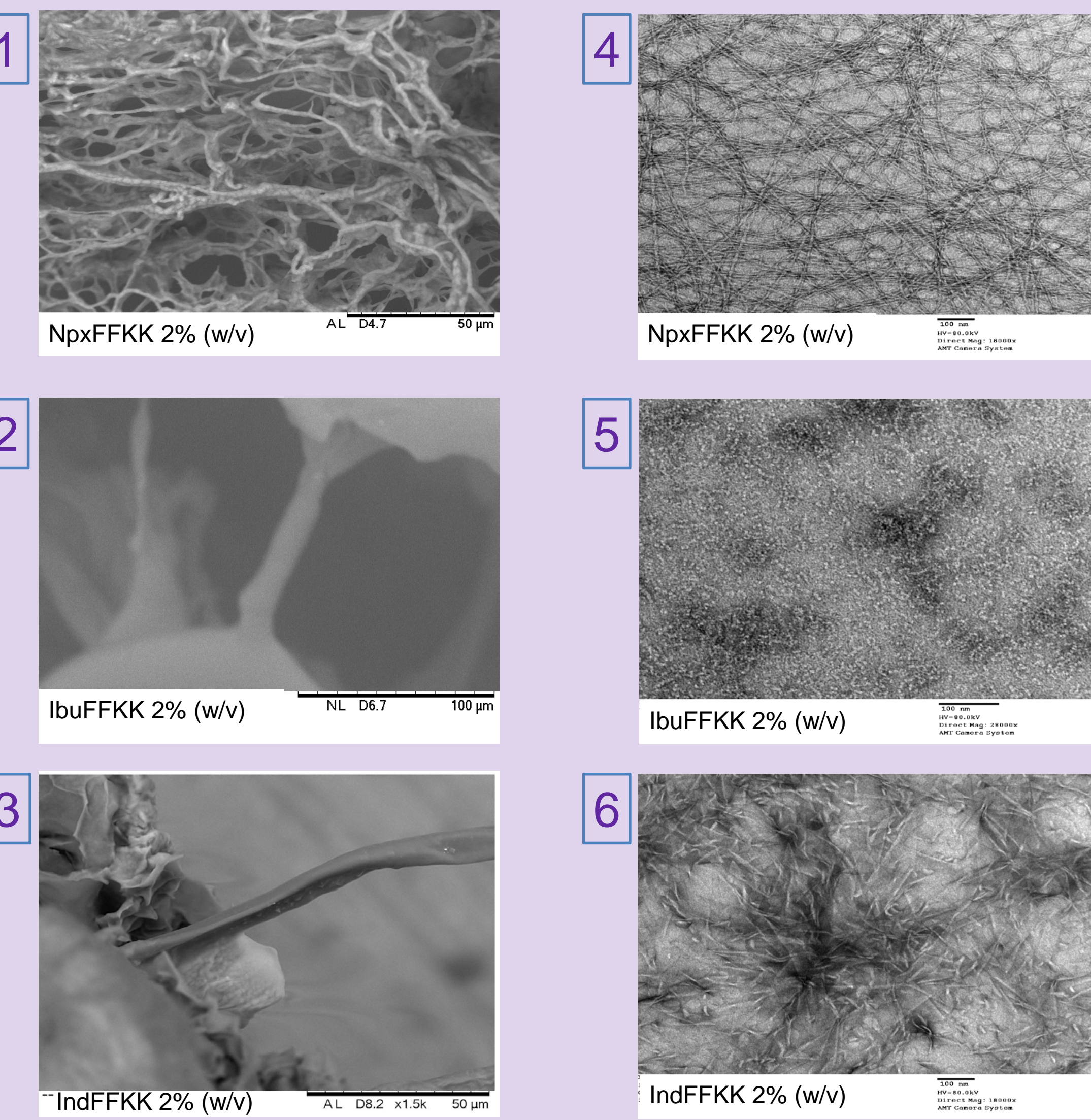


Fig.4. 1-3: cryo-SEM and 4-6: TEM images of the NSAID peptide hydrogels at 2% (w/v).

Conclusion

This investigation has shown that self-assembled NSAID conjugated peptide hydrogels may have potential clinically. Dual activity means that such molecules could act as novel candidates for wound dressings and medical device coatings. Further to this, pH triggered assembly and cytotoxic investigations conducted thus far indicates the potential for targeted activity at the necessary site of action. The next step will be to characterize further the biocompatibility and enzymatic stability of these molecules.

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